FIELD DETECTION AND IDENTIFICATION OF A BIOAEROSOL SUITE BY PYROLYSIS-GAS CHROMATOGRAPHY-ION MOBILITY SPECTROMETRY

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ABSTRACT

Improvements were made to a pyrolysis-gas chromatography-ion mobility spectrometry (Py-GC-IMS) stand-alone biodetector to provide more pyrolyzate compound information to the IMS detector module. Biological aerosols were disseminated at DRES, Alberta, Canada and the Py-GC-IMS was tested for its ability to detect the biological aerosols. Forty-two trials were conducted and a simple area calculation of the GC-IMS data domain biomarker peaks correlated with the correct bioaerosol challenge in 30 trials (71%). In another 7 trials, the status of an aerosol was determined to be biological in origin. Two additional trials had no discernible, unambiguous GC-IMS biological response, because they were blank water sprays. Reproducible limits of detection were at a concentration of less than 0.5 bacterial analyte-containing particles per liter of air (ACPLA). In order to realize this low concentration, an aerosol concentrator was used to concentrate 2000 liters of air in 2.2 minutes. The current series of outdoor trials has provided a platform to show that the Py-GC-IMS can provide information more specific than a biological or non-biological analysis to an aerosol when the time of dissemination is unknown to the operator. The Py-GC-IMS is shown to be able to discriminate between aerosols of a Gram-positive spore (BG), a Gram-negative bacterium (EH) and a protein (ovalbumin).

INTRODUCTION

Interest continues in the quest for a device that can detect the presence of biological material in a solid, liquid or aerosol sample. Among the interests of the military with respect to biological detection capabilities, operation scenarios require a need to address early warning of a foreign biological presence at perimeters of military posts, stations, airfields and battlefields. There is also a need to have biological detectors resident on military vehicles including tanks, soldier transport carriers, HUMVEEs, ships and aircraft. Pyrolysis-gas chromatography-ion mobility spectrometry (Py-GC-IMS) has been shown to be a

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useful tool for the detection of biological analytes in aerosols and injected liquid suspensions.[1-4] Either aerosol particulate or microliter amounts of a liquid suspension is introduced onto a quartz filter paper situated inside a pyrex tube. The tube is rapidly heated to 350^{o}C to produce pyrolyzate vapor from the solid sample. A portion of the sample is injected into a temperature-programmed, coil-shaped GC column assembly, and the eluate enters the ring-shaped ^{63}Ni ionization source of an ion mobility spectrometer. Ions are pulsed into a drift tube at close to atmospheric pressure by an ion voltage gate. The ions are detected at the end of the drift tube by a Faraday plate. A Py-GC-IMS device was tested under outdoor, prairie conditions with aerosol sprays of Bacillus globigii (BG), Erwinia herbicola (EH) and ovalbumin protein (OV) to determine the capability of the instrument to register the presence, possible identity and duration of an aerosol (when it is present and when it is not present).

EXPERIMENTAL MEHTODS

Testing of the ability of the Py-GC-IMS biodetector to respond to biological aerosol substances took place in the prairies of Defence Research Establishment Suffield (DRES), Alberta, Canada. A 916-liter per minute XM-2 aerosol collector/concentrator (upper part of Figure 1) was interfaced to the biodetector (lower section of Figure 1) by a Teflon-lined tube. The XM-2 has 50% and 26% efficiencies of aerosol collection for particulate with 5 and 2-micron diameters, respectively (unpublished data). The biodetector is housed inside a briefcase enclosure. A Met One particle counter was used to ascertain the total number of greater than or equal to one-micron diameter-sized particles at 8 s intervals (11 s display time), and this provided independent information on the total particulate burden in the air. The particulate burden includes biological and non-biological containing particles. The XM-2 is relatively larger (25 x 17 x 13 in.), heavier (approximately 66 lb) and uses more power (360 W) compared to the Py-GC-IMS (12 x 16 x 5 in., 15 lb including on-board computer and a peak power at 120 W with a running power of 48 W).

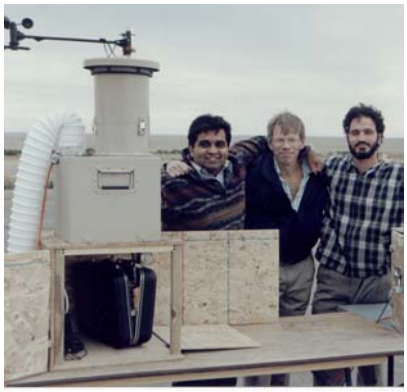

Figure 1. XM-2 Py-GC-IMS

Figure 2 provides a photograph of the briefcase-size Py-GC-IMS and the main components are outlined as follows: 1-aerosol inlet; 2-pyrex tube/pyrolysis source; 3-vacuum pump interface used to admit and deposit aerosol particulates onto the quartz filter; 4-high temperature three-way GC-injection valve; 5-patented[5] programmable, ring-shaped GC column (high temperature, stainless-steel GC column, 4 m x 0.5 mm with 0.25 micron methyl silicate coating); 6- ^{63}Ni ion source of the Chemical Agent Monitor (CAM)

ion mobility spectrometer; 7-dual diaphragm vacuum pump; 8- CAM ion mobility cell; 9- molecular sieve packs for scrubbing the ion mobility cell carrier gas; 10-molecular sieve packs to clean the ambient air; 11-15 V DC power in; 12-Visionbook Traveler 3000 computer; 13-electronic hardware and power supplies underneath the computer; 14-50-pin interface to PCMCIA data acquisition card; 15-heavy duty briefcase. A cycle of aerosol analysis begins with a 132 s collection of aerosol particulate (Figure 3) followed by a sample drying time with subsequent pyrolysis. The last 27 s of the previous cycle is also included in the 132 s total aerosol collection time. The collected particulate is dried for 11 s at 130°C followed by pyrolysis at 350°C. A two-second injection of pyrolysis products enters the GC column and analytical separation of the complex vapors ensues.

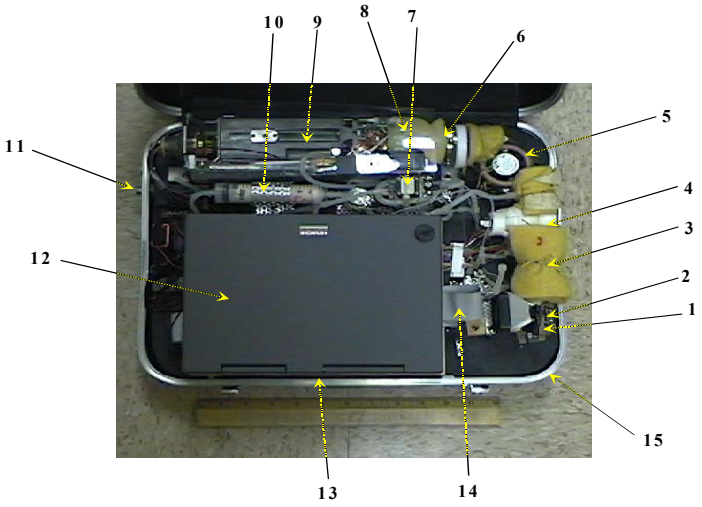

Figure 2. Py-GC-IMS; See Experimental for Details.

Figure 3 shows a 40 second period of time when the system is not collecting aerosols. The system is essentially blind to the environment during that period of time. The aerosol collector is turned on again during the GC elution phase that occurs during the last 27 s of the cycle. The computer in the Py-GC-IMS system was controlled at a distance of approximately 150 ft with PC ANYWHERE by a notebook computer via an Ethernet coaxial cable and a 10 MBPS PCMCIA card.

A vehicle-mounted Micronair agricultural sprayer assembly disseminated bioaerosols at approximately 100-250 m from where the Py-GC-IMS biological detector was placed on the prairie. The wet bacterial particles evaporated as they traversed the 100-250 m distance and resulted in particle diameters in the 0.7-10 µm range. An aerosol particle sizer was used to profile the particle size distribution. The test personnel enumerated bacterial aerosol particle counts.

Particle counters such as a Met One device measure total particles per liter of air (PLA), whereas an agar Petri dish, which is used to grow the bacterial aerosols, only measures viable bacteria-containing

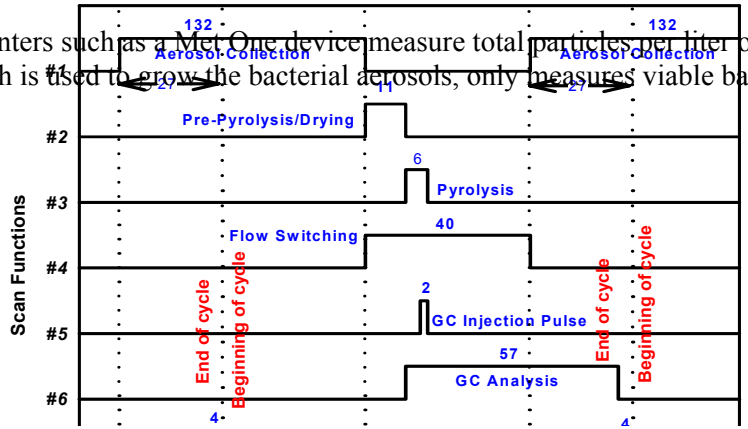

Figure 3. Sequence of events in a GC-IMS cycle.

particles, or ground truth. Thus, the Met One aerosol information is usually equal to or higher in particle counts than that of the Petri dish method. Operators of the biological detectors were kept in closed shelters with remotely controlled computers in communication with the outdoor-situated biodetectors. The identity and duration of a biological aerosol release was unknown to the test participants.

RESULTS AND DISCUSSION

Prior to the trials, the test director introduced known sprays at known times of the biological species that were used during the three-week series of aerosol releases at unannounced times. These known standard sprays followed the protocol as outlined in the Experimental Section. It was found that a certain peak for a biological substance was relatively unique in the GC-IMS data domain (data not shown). The following presents results of two windows over the three-week test period.

The analysis of the raw Py-GC-IMS spectra was accomplished by two different methods. The first one was a visual comparison of every 3 min 24 s GC-IMS data domain, and this was performed during the actual dissemination trials for all data domains in all 20 trial windows. This analysis did not rely on computer data reduction. A bioaerosol event is defined as the presence of one or more peaks in a GC-IMS data domain that are greater than 12% of that of the water reactant ion peak (RIP) signal intensity. The second method of analysis was performed with computer software during a period after the completion of all the trials.

Window 3. Figure 4 presents a comparison of the deliberately released bioaerosols during a 2 hr, 10 min window in an afternoon period and the biological ground truth for trials 5 (T5) and 6 (T6). Four

superimposed traces are shown in the top part of Figure 4, and three of them represent the GC-IMS peak area response of the bioaerosols in arbitrary units (right hand ordinate), for BG (●), EH (▲) and OV (■). A vertical series of these three symbols are present at each cycle, i.e., at every 3 min, 24 s. Each of the three symbols reflects the peak area or summation of the peak areas of a respective compound or compounds in the standard bioaerosol GC-IMS data domain (not shown). A confirmation or ground truth plot of the cultured bacteria over time is presented in the lower half of Figure 4. This information provides a real-time tracking of bacterial presence at the site of the Py-GC-IMS biodetector. The time between each ground truth point for OV is two minutes. In general during the three weeks of trials, there was the possibility that the aerosol may not have passed over the vicinity of the detector because of the unpredictable nature of the winds.

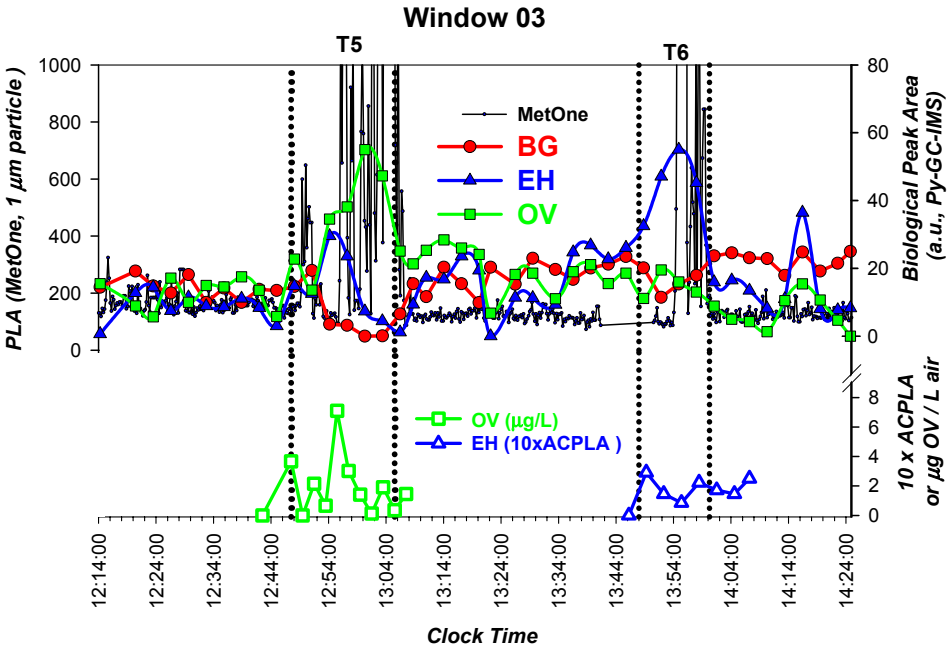

Figure 4. GC-IMS data and ground truth in Window 3.

Figure 3 shows that a Py-GC-IMS analysis has a 2 min 57 s delay in response to the actual presence of an aerosol. This is interpreted as that a recognizable biological response in the GC-IMS data domain actually occurs 2 min 57 s after the initial arrival of the aerosol over the device. For a test window, all the Py-GC-IMS cycles were computer-interrogated for the presence of the labeled peaks, or biomarkers. The time value of 2 min 57 s was subtracted from the clock time of the response of the biomarkers as observed in all of the cycles in a test window. This corrected time and respective peak areas for the three biological substances are plotted in Figure 4 as three symbols. There are two sets of vertical dotted lines in Figure 4. Each set represents a trial that consisted of an aerosol release. The earlier of each set of vertical dotted lines represents the time when the aerosol was turned on. The later vertical dotted line in each set represents the time when the test coordination personnel turned off the aerosol generator. The particular trial number in a

window is given above each set of dotted lines. Thus for window 3 in Figure 5, aerosol trials 5 and 6 were conducted, and the identities of the aerosols were OV and EH, respectively, as shown by the ground truth information in the lower half of the Figure. The shaded symbols represent the relative area(s) of the distinctive biomarker peaks as observed in the GC-IMS data domain. The open symbols refer to the ground truth data from the Petri dish bacterial growth or water bubbler collection of OV protein aerosols. The fourth superimposed trace in the upper part of Figure 4 is the Met One particle counter response. Significant aerosol event activity is observed within trials 5 and 6 from the Met One particle counter graph. Note that the signal is relatively more intense toward the later portion of both trials. The upper portion of trial 5 shows that the dominant Py-GC-IMS signal originates from biomarkers characteristic of OV protein, and OV was the biological substance used in the aerosol spray as observed in the lower left-hand side ground truth. A maximum of 8 µg OV/L air also is observed in the equivalent ground truth plot. A particle number distribution plot of OV aerosol during the trial (data not shown) yielded an average aerodynamic diameter of approximately 1 micron. The Py-GC-IMS system is a mass-based detector; therefore, the 2 and 5 µm particle diameters were used to characterize the amounts of particles introduced into the pyrolysis-processing module. The approximate amount actually deposited into the pyrolyzer quartz tube for cycle 15 is (8 µg/L)(916 L/min) (2.2 min) (0.26 to 0.50 efficiency) = 4.2-8.0 mg OV.

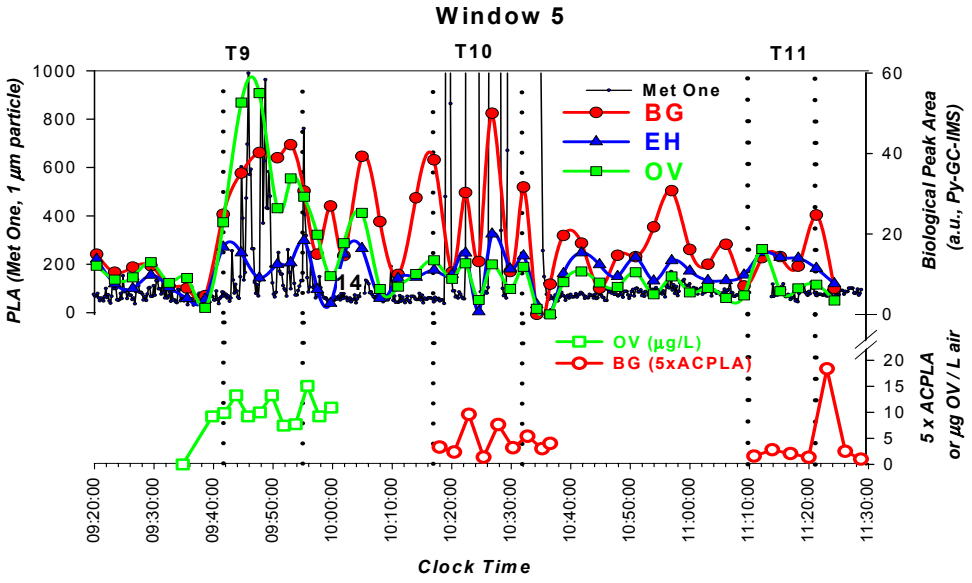

Figure 5. GC-IMS data and ground truth in Window 5.

Trial 6 indicates a clear Gram-negative EH bacterial aerosol by the GC-IMS data domain information, and indeed this was the actual disseminated aerosol as noted in the lower right-hand side ground truth data in Figure 4. The Met One response is off scale in both trials 5 and 6, however, a maximum total particle count of 1400 and 4500 PLA were present, respectively. The amount of viable EH aerosols collected in a cycle in trial T6 is approximately (0.4 particles/L)(916 L/min)(2.2 min)(0.26 to 0.50 efficiency) = 210-403 particles. The Py-GC-IMS instrument displayed good sensitivity in that less than 0.5 ACPLA of EH and less than 8 µg OV/L air were present in the environment in trials 6 and 5, respectively. Even at concentrations

of less than 0.5 of a bacterial-containing particle per liter of air, the Py-GC-IMS was able to detect the presence of the EH aerosol after sampling approximately 2000 L in a time frame of 132 s.

Window 5. Figure 5 presents the biological aerosol dissemination results for window 5, and three trials are observed. Trial 9 shows that the Met One background aerosol burden (less than 200 PLA) and OV GC-IMS signatures are clearly visible in the first half of the trial. This biological species was indeed released during the trial 9 timeframe and is confirmed in its ground truth plot (open squares). Trial 10 consisted of a very intense PLA dissemination at greater than 45,500 PLA. This was due to smoke aerosol interference. Mixed in with the smoke was an aerosol of BG spores. The GC-IMS peak area traces showed a strong BG bacterial response, and this is confirmed by the presence of BG ground truth data. The smoke component produced negligible interference because the RIP was clearly present (data not shown). This is an interesting observation because the high amount of smoke did not hinder the ability of the Py-GC-IMS to register the very low 1-2 ACPLA concentration of BG. A peak area analysis provides ambiguous results in trial 11 in Figure 5. In trial 11, a BG aerosol was released and for most of the trial, less than 0.5 ACPLA is observed from the ground truth data. However, at the end of the trial, approximately 4 ACPLA was present as shown in the ground truth plot in the lower right-hand side of Figure 5. The GC-IMS response mirrors these observations where at the end of the trial, an increase in GC-IMS response occurs at 11:21:00. It appears that the majority of the aerosol cloud contained a very low amount of BG over the Py-GC-IMS point detection system, and at the end of the trial, a relatively significant amount of BG aerosol was exposed to the system. Two intense signals for BG occur between 10:06:00 and 10:56:00. Because no agar plate ground truth or independent measurements were taken, these events can be considered either as true positives or false positives. It is possible that reaerosolization of ground-containing BG spores occurred to result in the earlier event. For the later BG event, it is possible that a shift in wind direction directed the BG cloud, disseminated in T10, back over the biodetection system. Both events were visually observed in the separate GC-IMS data domains (data not shown).

CONCLUSIONS

There were 20 windows with a total of 42 aerosol trials. The peak area analyses of the GC-IMS data domains (not shown) correctly provided the identity, from three possible choices, of the aerosol in 30 of the 42 trials (71%). The peak area analyses labeled an aerosol response as biological in origin for another 7 trials. This particular category includes analyses where the wrong biological substance was chosen, i.e., BG was recorded while OV was actually present in an aerosol spray event. Two other trials witnessed the release of only a water spray, and for one of the trials, the GC-IMS data domains showed signals which could not be ascertained as either biological or non-biological (data not shown). This was recorded as an unknown aerosol event. For a second water spray, a complete absence of an aerosol event was noted. For the latter, no report was presented because an aerosol event was not recorded. Thus, these two trials showed a satisfactory match between experimental and actual results. In another trial, the GC-IMS indicated a true negative response. Therefore, 38 of 39 trials provided responses better than or equal to the presence of a biological or non-biological aerosol. There was one trial where the GC-IMS analysis determined an aerosol event of unknown origin even though it actually contained a biological substance. This can be labeled as a detection of an aerosol event by the instrument. There were two false positive trials (5%) for biological substance presence as related by the instrument, because in reality, one trial consisted of a water spray and the other trial was that of a smoke aerosol.

No false negative trials occurred. No chemicals, liquids, solutions or bottled gases are required to operate the system, and it is essentially solid state in nature. Consumables (cost, frequency of replacement) include the quartz filter paper (less than one cent, 12 hours), GC column ($80, 1 year), quartz tube ($8, 100 hr of continuous operation) and molecular sieves ($5, 2 months). The relative increase in information production coupled with the capability to distinguish between three fundamental classes of biological substances from a 15 lb, hand-portable Py-GC-IMS system with relatively convenient logistics represent an attractive concept for the detection of biological substances.

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